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2077 In Vitro Reconstruction of the Conjunctiva. C.F. Elking, B.E. Hull, E.P. Purdy, and J.D. Bullock. Biological Sciences Department and School of Medicine, Wright State University, Dayton, OH 45435.

We have adapted the procedure for construction of bilayered skin equivalents to reconstruct conjunctiva in vitro. Fibroblasts, obtained by outgrowth from biopsies of either human or rabbit conjunctiva, were combined with medium, serum, and rat tail tendon collagen. A suspension of either rabbit or human conjunctival epithelial cells were layered over the contracted collagen matrix at a concentration of less than 50,000 cells/cm^2. The human conjunctival epithelial cells formed a continuous covering, 2 to 3 cell layers thick, within 14 days after their addition to the collagen matrix. Preliminary immunofluorescent studies have demonstrated the production of a continuous band of material that binds primary antibodies to human type IV collagen, suggesting the formation of a basement membrane. Bilayered matrices constructed using rabbit cells have been successfully transplanted to the inner surface of rabbit eyelids, suggesting that these cells have retained their functional properties in vitro.
(Supported by NIH grant R01 AR-39297 to BEH.)

2078 Growth and rDNA Protein Production in an Improved Serum-free Medium Formulation. S.E. Gorfien[1], M.L. Tilkins[1], D. Judd[1], I. Boime[2], M. Pixley[2] and S.A. Weiss[1]. [1]GIBCO/LTI, Grand Island, N.Y. and [2]Washington University School of Medicine, St. Louis, MO.

Chinese Hamster Ovary (CHO) cells are widely used for expression of rDNA proteins because of their low rate of spontaneous transformation. The numerous disadvantages associated with the use of serum as a medium supplement led to the development of serum-free formulations for CHO cells. Many of these formulations were either designed for small scale monolayer cultures or were not fully optimized. By reducing the major protein components of GIBCO CHO-S-SFM and supplementing with other nutrients determined to be necessary by HPLC and other analytical means, an improved medium, GIBCO CHO-S-SFM II was derived. Total protein content of CHO-S-SFM II is less than 100 µg/mL. The medium is designed specifically for growth of CHO cells and expression of rDNA proteins in suspension culture. CHO cells that have been adapted to suspension culture in CHO-S-SFM II have been demonstrated to reach maximal cell densities in excess of 3 x 10^6 viable cells/mL. Comparison of CHO-S-SFM II with four other commercially available serum-free media for CHO cells showed superior growth of cells in CHO-S-SFM II. Production of rhCG by CHO cells in CHO-S-SFM II equaled or exceeded that observed in the earlier formulation, CHO-S-SFM. Successful cryopreservation and recovery of cells in this medium was demonstrated. The low protein content of CHO-S-SFM II facilitates downstream processing of recombinant proteins, thereby reducing the cost of the final product. Low endotoxin levels make this medium desirable from a regulatory standpoint for production of therapeutic proteins.

2079 Human ectocervical epithelial cultures grown on porous filter: Electrical and morphological characteristics. G. I. Gorodeski, M. F. Romero, W. H. Utian, Departments of Reproductive Biology and Genetics, Case Western Reserve University School of Medicine, Cleveland, OH 44106. (Spon. by G. R. Bright)

The epithelium of the human female reproductive tract regulates in vivo the luminal fluid content and its pH, and therefore plays a major role in sperm and ovum migration and in the process of implantation. The purpose of the present study was to develop a culture system of cells derived from that epithelium, and grown on a filter support to enable control of the luminal and basolateral solutions. We used human ectocervical epithelial cells, (hECE) and an HPV-16 immortalized line (ECE-16/1) derived from these cells, both of which have been shown to resemble the native ectocervical epithelium in that they retain in-vitro their typical morphology, stratify and produce envelopes and express cytokeratins but not vimentin. Cells were grown on a filter membrane coated with either Matrigel or poly-L-lysin. Confluent cultures showed time-related decrease in conductance, which reached a plateau of 35mS·cm^{-2} after four days in culture. Transepithelial permeability to low molecular weight (≈500) molecules ranged 14-35·10^{-5}cm·sec^{-1} and was 3-5 fold lower compared to controls. Transport experiments with fluorescent dextrans revealed no fluxes of 40KD and 70KD dextrans and low permeability (3·10^{-6}cm·sec^{-1}, 1/5 of control) to 10KD dextrans. Cultures mounted on a modified Ussing chamber demonstrated a µM amiloride-sensitive (apical) positive Isc which was augmented by µM ATP and reversibly inhibited by apical DPC. TEM revealed formation of tight junctions and villi-like structures facing the luminal aspect. These results indicate the establishment in-vitro, for the first time, of electrically confluent, although leaky, and polarized human ectocervical epithelium.

2080 Preliminary characterization of a teleost liver cell line derived from a chemically induced hepatocellular carcinoma isolated from the desert topminnow *Poeciliopsis lucida*. L. A. Ryan, C. N. White, L. E. Hightower Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT 06269.

Cell lines derived from teleosts and other cold blooded vertebrates can provide useful alternatives to traditional mammalian cell culture model systems. PLHC-1 cells, an epithelial cell line derived from a serially transplantable hepatocellular carcinoma, have now been in culture for over 200 generations. They are routinely grown at 30°C in MEM with 5% fetal bovine serum with a doubling time of approximately 38 hours, and are subcultured using trypsin-EDTA. Cultures are easily stored in liquid nitrogen using either 10% glycerol or 10% DMSO as cryoprotective agents. Approximately 33% of the cells are diploid (2n=48) but the modal chromosome number is 49. PLHC-1 cells have been successfully transplanted back into *P. lucida* where they form tumors, and are capable of forming colonies in soft agarose. Ultrastructural studies show the presence of tight junctions, basolateral interdigitations and abundant glycogen granules. One of the most important characteristics of these cells is their ability to metabolize and process metabolism-mediated cytotoxicants such as 7, 12-dimethylbenz(a)anthracene; they have been shown to possess cytochrome P450 activity inducible by 3, 3',4, 4'-tetrachlorobiphenyl (M. E. Hahn et al.) or Arochlor 1254 (H. Babich et al.) making them very useful for studying both the mechanisms of induction and for use in *in vitro* cytotoxicity assays. PLHC-1 cells also exhibit a very strong cellular stress response, producing 3 major classes of stress proteins that together account for almost 50% of the total protein synthetic capacity of heat shocked cells.

2081 Proliferation of Differentiated Urothelium In Vivo on Biodegradable Polymer Scaffolds. A. Atala, J.P. Vacanti, C.A. Peters, P.D. Guthrie, A.B. Retik, J.Mandell, and M.R. Freeman Pediatric Urology Research, Department of Surgery, Children's Hospital and Harvard Medical School, Boston, MA 02115

We have developed a novel system for the delivery and growth of rabbit urothelial cells in athymic nude mice on polyglycolic acid polymers (PGAP). The purpose of this study was to determine the proliferative potential and differentiation state of rabbit urothelium in PGAP scaffolds implanted in host animals for 5 to 50 days. Urothelial cells were collected by collagenase treatment of ligated rabbit bladders or by explant of mucosal surfaces directly onto PGAP scaffolds. After a brief culture period the cell-polymer scaffolds were implanted into the mesentery, omentum or retroperitoneum of athymic mice. Animals were sacrificed at 5, 10, 20, 30 and 50 days after implantation and implants were retrieved and evaluated using histologic, immunocytochemical and immunoblot methods. Vascular ingrowth was evident in most implants by 5 days. Hematoxylin and eosin staining and anticytokeratin immunohistochemistry demonstrated the presence of epithelial cells in some implants at all time points. Urothelial cell proliferation was suggested by the formation of epithelial sheets at later time points and was confirmed by in vivo BrdU labeling and anti-BrdU immunodetection. Maintenance of urothelial differentiation was indicated by immunoblot detection of urothelial-associated cytokeratins and immunohistochemical detection of uroplakin, a urothelial cell surface protein. Progressive degradation of the PGAP occurred coincident with expansion of the urothelial cell population. These findings suggest that cell populations of normal urothelium can be expanded by cultivation on PGAP scaffolds in vivo.

2082 Muscarinic and Purinergic Receptor-Stimulated Elevation of $[Ca^{2+}]_i$ and Increase in Short-Circuit Current Across Immortalized Rat Parotid Acinar Cells. S.P. Soltoff and D.H. Jefferson. Dept.of Physiology,Tufts Univ. School of Medicine, Boston,MA 02111

To develop a parotid cell line to study signal transduction and ion transport events, we immortalized rat parotid acinar cells by transducing SV40 large T antigen into cells using retroviruses.The resulting cell line(RPG1/SV40, rat parotid gland 1/SV40 transformant) forms monolayers and has tight junctions, which allows measurements of transepithelial electrophysiology. For details of transformation and cell biology, see S.A.Grubman et al. abstract. In this study we investigated alterations of $[Ca^{2+}]_i$ (intracellular free calcium, measured using Fura2) and I_{SC} (short circuit current, measured using Ussing-type chamber and voltage clamp) in response to carbachol and extracellular ATP, agonists which activate muscarinic and purinergic receptors, respectively, in the parent cells. $[Ca^{2+}]_i$ was measured in cell suspensions using a fluorometer and in single cells using a fluorescence microscope. Cells responded to both agonists with large elevations in $[Ca^{2+}]_i$. Single cell measurements of $[Ca^{2+}]_i$ indicated that 86% (31/36) of the cells that responded to ATP also responded to carbachol, demonstrating that both types of receptors were present on each cell. Both agonists stimulated an increase in I_{SC} (≈3 µA/cm^2), and the Ca^{2+} ionophore ionomycin produced a large increase (≈ 9 µA/cm^2). I_{SC} may measure a net transepithelial Cl^- secretory current. These studies indicate that RPG1/SV40 cells are an excellent model system to study parotid acinar cells, including processes involved with signal transduction and transepithelial ion movements.
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